

Coagulation studies in rheumatic heart disease

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SUMMARY The clotting characteristics of pulmonary and systemic blood were studied in 10 patients with chronic rheumatic mitral valve disease complicated by atrial fibrillation and in seven patients with aortic valve disease in sinus rhythm. A haemostatic basis for the association of rheumatic mitral valve disease with thrombotic emboli was sought.

Both groups of patients showed differences in platelet function between pulmonary and systemic arterial blood. In patients with mitral valve disease aggregation of platelets was significantly greater in pulmonary than in systemic arterial blood at rest; the converse was true during exercise. In aortic valve disease platelet aggregation was greater in systemic than in pulmonary arterial blood at all times. Only the patients with mitral valve disease showed changes in blood coagulation during passage through the lungs and left heart; there was a small but statistically significant shortening in partial thromboplastin time in systemic as compared with pulmonary arterial blood both at rest and during exercise. Similarly, the effects of exercise on the various haemostatic factors measured were largely confined to the patients with mitral valve disease; in these patients exercise stimulated an increase in factor VIII in pulmonary arterial blood and an increase in platelet adhesiveness and aggregability in left heart blood. These changes provide a basis for the suggestion that in patients with rheumatic mitral valve disease, unlike those with aortic valve disease, there is an increased thrombotic tendency in blood in the left heart which is particularly pronounced during exercise.

Systemic emboli are a serious hazard of rheumatic mitral valve disease.^{1,2} The risk does not appear to be related to the type of valvular deformity,³ or haemodynamic derangement.⁴ Though relevant, few studies have been reported of possible abnormalities in the clotting characteristics of blood as it passes across the damaged mitral valve. The following study was undertaken to determine whether such abnormalities exist.

Subjects and methods

Seventeen patients were studied. Ten, including six women, had moderate or severe mixed mitral valve disease complicated in all by chronic atrial fibrillation. Seven patients, including four women, with mild aortic valve disease in sinus rhythm furnished a control population. Clinical diagnosis in all was confirmed by angiographic and haemo-

dynamic studies. No patient was in congestive heart failure, none had a history of embolic episodes, and none had taken anticoagulants. All gave their consent for the study which was agreed by the hospital ethical committee.

DESIGN OF STUDY

(a) *In vitro* studies

These were undertaken to determine the effect of the passage of blood through the catheters used in the *in vivo* study. Peripheral venous blood was taken from eight healthy volunteers (four men, four women) into plastic syringes without anticoagulation. Blood was taken into three syringes; from the first syringe blood was transferred direct to 1/9 volume 3.8 per cent sodium citrate, and from the second and third syringes was passed, respectively, through the pulmonary (8F US Catheter Co. Ltd.) or the arterial (75 × 0.2 cm Portex Ltd.) catheter. The first 5 ml blood passing through each catheter were discarded and the remainder collected in the appropriate tubes for platelet and coagulation factor measurements.

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(b) *Comparison of blood clotting variables in pulmonary and systemic arterial circulations*

Measurements were made at the time of routine diagnostic haemodynamic evaluation. Blood samples were withdrawn simultaneously from the pulmonary and systemic arteries at rest and at the end of two minutes of submaximum supine bicycle exercise. Pulmonary arterial blood was withdrawn through a woven Dacron catheter (8F US Catheter Co. Ltd.) and aortic blood through a nylon catheter (75 × 0.2 cm Portex Ltd.). No anticoagulants were used; catheters were kept patent by saline infusion.

The first 10 ml blood withdrawn from each catheter were discarded. Samples were then taken into plastic syringes and mixed with heparin (40 IU/10 ml), for platelet adhesion studies, with 1 per cent tranexamic acid for fibrin related antigen measurement, with EDTA for platelet counts, and with 1/9 volume 3.8 per cent sodium citrate for the remaining tests.

The citrated blood was spun at 250 g for 15 minutes to obtain platelet rich plasma which was drawn off with a siliconised pipette. The remainder of the blood was spun at 2500 g for 20 minutes at 4°C to obtain platelet poor plasma.

Platelet counts were performed on a Coulter thrombocounter. Coagulation studies were performed by the following methods:

Activated partial thromboplastin time⁵; fibrinogen⁶; plasminogen^{7,8}; urokinase sensitivity⁹; euglobulin lysis time⁹; fibrinogen related antigen¹⁰; factor VIII¹¹; platelet adhesiveness¹²; platelet aggregation¹³; platelet factor 3.¹⁴

Platelet aggregation was measured in response to adrenaline (acid bitartrate, Sigma Chemical Co., St. Louis), to ADP (Sigma Chemical Co., St. Louis), and to soluble collagen.¹⁵ These agents were diluted in tris-buffered saline pH 7.4 and added to the platelet rich plasma in amounts to give final concentrations of 5 µmol adrenaline and 0.5, 2.0, and 4.0 µmol ADP. A quantitative assessment of platelet aggregation was made by measuring the slope of the changes in light transmission (IR), the amount of light transmission three minutes after addition of the aggregation agent (T₃), and the maximal light transmission achieved (T max) within three minutes.

Results

CATHETER EFFECTS ON BLOOD CLOTTING VARIABLES

The results are summarised in Table 1. No statistically significant difference was noted in any of the indices studied between the blood that was

Table 1 *Comparison of effects of passage of non-anticoagulated peripheral venous blood from healthy volunteers through pulmonary and arterial catheters (mean values ± standard error of mean)*

Variables	Before catheter transit	Pulmonary catheter	Arterial catheter
Partial thromboplastin time (s)	30.3 ± 1.1	31.9 ± 1.8	30.4 ± 1.2
Platelet count (× 10 ⁹)	241 ± 17.7	247 ± 18.7	236 ± 14.9
Platelet aggregation to			
0.5 µmol ADP IR	25.1 ± 3.6	27.7 ± 4.8	26.8 ± 4.1
T ₃	3.1 ± 1.7	3.3 ± 1.3	2.7 ± 1.3
T max	7.6 ± 1.5	8.0 ± 1.8	7.8 ± 1.6
2.0 µmol ADP IR	73.3 ± 3.9	74.8 ± 6.7	72.3 ± 4.7
T ₃	52.6 ± 6.0	49.1 ± 6.4	51.1 ± 5.1
T max	55.0 ± 3.7	47.8 ± 6.7	52.3 ± 4.1
collagen IR	105.8 ± 5.5	100.7 ± 4.6	96.2 ± 4.6
T ₃	63.9 ± 1.2	63.4 ± 0.9	61.1 ± 2.0
4 µmol adrenaline IR	26.6 ± 4.5	31.6 ± 4.9	27.3 ± 4.9
T ₃	51.4 ± 3.7	49.0 ± 5.1	49.7 ± 4.6
Factor VIII	132 ± 8.7	125 ± 10.9	128 ± 10.9
Platelet adhesion (%)	46.4 ± 7.5	48.0 ± 8.3	44.4 ± 7.5
Plasminogen (casein units/ml)	3.1 ± 0.2	3.3 ± 0.2	3.1 ± 0.2
Fibrinogen (g/l)	1.9 ± 0.2	2.0 ± 0.1	1.9 ± 0.1
Euglobulin lysis time (min)	332 ± 50.0	329 ± 47.8	319 ± 48.9

No statistically significant difference was noted between the three sets of determination.

and was not passed through arterial or pulmonary catheters.

COMPARISON OF HAEMOSTATIC FACTORS IN PULMONARY AND SYSTEMIC ARTERIAL BLOOD

Factors in which there was a statistically significant difference are detailed in Table 2.

In mitral valve disease

At rest: there was a small decrease in activated partial thromboplastin time and a decrease in platelet aggregation in aortic as compared with pulmonary arterial blood.

During exercise: the small atrioventricular difference in PTT was maintained accompanied by a significant decrease in platelets and an increase in platelet aggregation (to 2.0 µmol ADP T max) in aortic as compared with pulmonary arterial blood.

In aortic valve disease

At rest: there was a significant increase in platelet aggregation in aortic as compared with pulmonary arterial blood.

During exercise: the increase in platelet aggregation in aortic blood was maintained.

CHANGES IN HAEMOSTATIC FACTORS INDUCED BY EXERCISE

Factors in which there was a statistically significant

Table 2 Statistically significant changes in haemostatic measurements: effects of transit through pulmonary circulation and left heart—mean values are presented

Variable	Mitral valve disease				Aortic valve disease			
	Rest		Exercise		Rest		Exercise	
	Pulmonary	Systemic	Pulmonary	Systemic	Pulmonary	Systemic	Pulmonary	Systemic
PTT (s)	36 ± 2	34 ± 1*	35 ± 2	33 ± 1‡	—	—	—	—
Platelet count ($\times 10^9$)	—	—	231 ± 37	202 ± 28*	—	—	—	—
Platelet aggregation to 0.5 μ mol ADP	IR	48 ± 11	35 ± 10‡	—	—	42 ± 8	56 ± 8*	—
	T _s	5.4 ± 1.4	3.1 ± 1.1†	—	—	—	11.8 ± 2.7	14.7 ± 2.8‡
2.0 μ mol ADP	T max	—	33 ± 5	44 ± 6*	—	—	—	—
	T _s	—	—	—	42 ± 9	55 ± 7*	—	—
Collagen	IR	81 ± 8	63 ± 9*	—	—	—	—	—
	T _s	67 ± 3	55 ± 6*	—	—	—	—	—
Adrenaline	T _s	—	—	—	30 ± 4	39 ± 6*	—	—

* $p < 0.05$; † $p < 0.02$; ‡ $p < 0.01$.

difference are detailed in Table 3.

In mitral valve disease

Exercise was associated with an increase in factor VIII in pulmonary arterial blood and an increase in platelet adhesion and aggregation in systemic arterial blood.

In aortic valve disease

Exercise was associated with an increase in platelet aggregation in pulmonary arterial blood; there were no changes in any of the other haemostatic factors measured in either pulmonary or aortic blood.

Discussion

These observations demonstrate a number of differences between the clotting factors in the pulmonary and systemic arterial blood of patients with advanced mitral valve disease and those in a control population of patients with mild aortic valve disease. As shown in Table 1, passage through the different catheters produced only trivial and statistically insignificant changes in the haemostatic function tests.

The most pronounced changes found in the patient studied were seen in platelet function. At rest platelet aggregation was less in systemic than in pulmonary arterial blood in patients with mitral valve disease; in patients with aortic valve disease the converse was true. During exercise both groups showed an increase in platelet aggregation in systemic as compared with pulmonary arterial blood, and in the patients with mitral valve disease this was accompanied by a diminution in aortic platelet count. Exercise was responsible for a pronounced increase in platelet adhesion and aggregation in aortic blood in mitral valve disease; in contrast, changes in aortic valve disease were confined to a limited increase in aggregation in pulmonary blood.

Before attempting to interpret these complex changes it is well to examine the limitations imposed by the design of the study. None of the patients with mitral valve disease had had emboli; the haemostatic factors in this cohort may, therefore, be quite different from those in a group of patients who had suffered such an event. The control group of patients with aortic valve disease was chosen for ethical reasons. However, their valve lesion was

Table 3 Statistically significant changes in haemostatic measurements: effects of exercise—mean values are presented

Variable	Mitral valve disease				Aortic valve disease			
	Pulmonary arterial		Systemic arterial		Pulmonary arterial		Systemic arterial	
	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
Factor VIII	107 ± 5	120 ± 7*	—	—	—	—	—	—
Platelet adhesion (%)	—	—	59 ± 7	63 ± 6†	—	—	—	—
Platelet aggregation to 0.5 μ mol ADP	IR	—	35 ± 10	57 ± 12‡	42 ± 8	65 ± 12*	—	—
	T _s	—	3.1 ± 1.1	8.0 ± 2.3‡	—	—	—	—
	T max	—	12.8 ± 3.4	21.5 ± 4.2‡	—	—	—	—
Collagen	IR	—	63 ± 9	95 ± 8‡	—	—	—	—
	T _s	—	55 ± 6	68 ± 2†	—	—	—	—

* $p < 0.05$; † $p < 0.02$; ‡ $p < 0.01$.

mild, all were in sinus rhythm, and their clinical state was such as to warrant their inclusion as reasonable control population. It could also be argued that a higher workload or longer periods of exercise would have induced more pronounced changes. This may indeed be true, but both were at the limits ethically justifiable during investigation of patients with mitral valve disease. Finally, it must be emphasised that the pulmonary systemic arterial differences in blood clotting factors measured describe only composite changes; they allow no separation of the particular effects of transit through the lesser circulation from those occurring during passage through the left atrium and over the damaged mitral valve.

Despite these design limitations, the results are interesting. The reduction in platelet aggregation after passage through the pulmonary circulation and left heart at rest in patients with mitral valve disease contrasts with the reverse situation in aortic valve disease. However, during exercise which can be expected to increase platelet stickiness,¹⁶ there was an increase in platelet aggregation in systemic as compared with pulmonary arterial blood in both groups; in the patients with mitral valve disease there was additionally a coincidental reduction in the systemic arterial platelet count. The interpretation of these changes in pathological terms is speculative. It can be reasoned that in the patients with mitral valve disease at rest, many of whom have shortened platelet survival,¹⁷ the more sticky platelets were sequestered in the pulmonary blood vessels or left heart during the retarded passage of blood through the lesser circulation. There is a substantial incidence of pulmonary arterial intimal damage and atheroma in patients with pulmonary hypertension caused by mitral valve disease; such intimal lesions could be expected to form a nidus for deposition of the most sticky platelets. There is no large platelet pool in the lesser circulation,¹⁸ so that the more rapid flow of blood through the lungs during exercise could be expected to reverse this trend, as was observed. However, these speculations are at variance with the observations of Kallinkos-Maniatis,¹⁹ who found a significantly *greater* platelet count in systemic as compared with central venous blood in patients with a range of cardiac diagnoses. The difference in our findings could possibly be explained by differences in the techniques of measurement used and the patients in the two studies.

Recently published observations^{20 21} have shown that in a number of animal species the lungs are the site of continuous generation of prostacyclin (PGI₂) the most potent inhibitor of platelet aggregation so far described, and that vigorous

hyperventilation increases release from the lungs of a platelet disaggregating agent. Piper and Vane²² showed that injection of particles into guinea-pig lungs led to release of prostaglandins, except when the injections led to an increase in pulmonary arterial pressure when there was no detectable release of prostaglandins. It may be that our studies in man can be explained on the basis that the aortic valve disease 'control' patients mimic the general animal response cited above and that mitral valve disease patients with their damaged lungs and pulmonary arterial hypertension at rest mimic the guinea-pigs and only behave as the aortic valve disease patients do after the stimulus of exercise and the associated increase in ventilation.

The only other difference in haemostatic changes between the two groups was in the blood coagulation screening test, PTT. Activated PTT was less in systemic than in pulmonary arterial blood both at rest and during exercise in patients with aortic valve disease. In the group with mitral valve disease exercise also increased factor VIII activity, confirming other reports.²³ Again, though the pathological interpretation of these findings is speculative, the increase in factor VIII activity during exercise and the reduction in PTT during passage of blood through the lesser circulation could possibly be expected to increase the tendency to thrombus formation, particularly in atheromatous pulmonary vessels or left atrium, the seat of endocardial damage or excessive blood stasis.

These studies give no clue as to the mechanisms involved in these platelet and blood coagulability changes in patients with mitral valve disease, but they prompt an interesting speculation. The prime characteristic of impaired cardiac function is reflex activation of the sympathoadrenal system and a raised concentration of catecholamines in the plasma.²⁴ These changes are particularly noticeable in patients with mitral valve disease and increase during exertion. Such sympathoadrenal activation can be expected to stimulate the breakdown of energy-rich ATP to ADP in the tissues and blood cells and thus enhance the adhesiveness of platelets and their tendency to aggregation.

Whatever the mechanisms involved, our findings are consistent with the fact that patients with mitral valve disease do indeed have an increased tendency to platelet deposition and thrombin formation followed by fibrin deposition in the lesser circulation and left heart. This is not to imply that such changes are the primary cause of clotting events in these patients. It is much more likely that the tardy blood flow, the high pressures in the pulmonary vessels and left atrium, and the intimal damage in these vascular compartments are the basic pathogenic

factors. However, our results are fully compatible with the haemostatic changes that could be expected in such a situation. They also give theoretical support to the practical therapeutic observations that in mitral valve disease anticoagulants reduce the incidence of arterial embolism,^{1 25} and that antiplatelet agents do so in patients after mitral valve surgery.²⁶ Clearly, more work is necessary, and results give impetus for such endeavours.

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